



Effect of *Chrysanthemum indicum* Against Cisplatin-Induced Nephrotoxicity

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ABSTRACT

The present study was carried out to evaluate the effect of ethanol extract of flowers of *Chrysanthemum indicum* against Cisplatin-induced nephrotoxicity. Nephroprotective activity was studied in male Wistar Albino rats. Nephrotoxicity was induced by Cisplatin at a dose of 6mg/kg i.p., on day 1. Nephroprotector activity of the ethanol extract was tested at two dose levels i.e., 250mg and 500mg/kg body weight. Nephroprotector activity was assessed by determination of serum marker levels, urinary functional parameters and lipid peroxidation activity (LPO) in kidney homogenate. Histological studies were conducted. In the present study, Cisplatin induced nephrotoxicity was characterized by significant elevation of serum markers levels, increased urinary protein excretion, raised LPO levels, reduced creatinine clearance. Extract of flowers of *Chrysanthemum indicum* had significantly prevented the renal injury by decreasing the levels of serum total proteins, urinary total proteins, lipid peroxidation levels and increasing the creatinine clearance. Histological studies substantiated the above results. Significant nephroprotective activity of ethanol extract of flowers of *Chrysanthemum indicum* may be due to its antioxidant properties and the effect was dose dependent. The present study provides the corroborative scientific evidence for the folklore use of *Chrysanthemum indicum* in urinary troubles.

Keywords: *Chrysanthemum indicum*; Cisplatin; Serum markers

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INTRODUCTION

Cisplatin is one of the most effective anticancer drugs administered to treat a variety of cancers¹⁻⁵. Although effective, Cisplatin is associated with many adverse drug reactions, such as renal damage, gastrointestinal dysfunction, ototoxicity, and peripheral nerve toxicity. Nephrotoxicity in particular is a major complication and a dose-limiting factor for cisplatin therapy⁶. The possible involvement of peroxidative damage caused by a reactive oxygen species (ROS) has been suggested in the pathogenesis of Cisplatin-induced renal failure⁷. A series of therapeutic agents were experimentally evaluated against Cisplatin-induced nephrotoxicity but none of them exhibited effective protection against Cisplatin-induced renal damage. Literature reveals that number of medicinal plants showed significant protection against Cisplatin-induced nephrotoxicity. Upon thorough literature survey, it was evident that plants with anti oxidant principles exhibited significant nephroprotection. *Chrysanthemum indicum* is one such plant which is rich in phenolic compounds and tribal people of Rayalaseema⁸, Andhra Pradesh use these flowers to treat urinary troubles but till date no systematic study has been carried out on this. Hence, the present study was designed to screen the effect of the ethanol extract of flowers of *Chrysanthemum indicum* for its nephroprotector activity.

MATERIALS AND METHODS:

Collection of plant material:

Flowers of *Chrysanthemum indicum* were collected from local market of Chittoor district and authenticated by Dr. Madhava chetti, Botanist, Department of Botany, S.V University, Tirupati and voucher specimen was deposited in S.V.U. Botany department.

Preparation of ethanolic extract:

Flowers of *Chrysanthemum indicum* were shade dried and powdered in Wiley mill. 60 grams of powder was subjected to Soxhlet extraction with petroleum ether for 12hrs; then the powder was air dried and again subjected to extraction with alcohol for 12 hrs by Soxhlet apparatus. The extract was then concentrated under vacuum to get semisolid residue. The procedure was repeated to obtain required amount which was used to conduct phytochemical and pharmacological studies.

Column chromatography of the ethanol Extract of flowers of *Chrysanthemum indicum*:

20 grams of ethanol extract of flowers of *Chrysanthemum indicum* was suspended in and adsorbed on Silicagel on 60-120 mesh size. Graded elution was done with pure and mixed solvents comprising of Petroleum ether and Chloroform. 5mg of a colour solid was obtained at

20-30 fraction with 100% Petroleum ether, A Semisolid mass of oily consistency (5mg) was obtained at 5:5 (Pet.ether: chloroform) 66-71 fraction.

Pharmacological studies

Animals :

Healthy Wistar adult male albino rats between 2 - 3 months of age and weighing about 150-200g had been used in the present study. Animals were acclimatized to the lab environment and provided with abundant food and water. This study was approved by Institutional Animal Ethical committee (no: 1677/PO/a/12/CPCSEA).

Treatment protocol :

Animals were divided into seven groups of 6 animals each and they were put on the following treatment schedule:

Group	Treatment	Day of biochemical estimations	Purpose
I	Normal control - received only vehicle (2% Gum acacia)	5 th , 15 th & 16 th	Normal control
II	Curative control - cisplatin(6mg/kg.,i.p) on day1+ vehicle 10 days (6 to 15)	5 th & 16 th	To serve as control for groups III & IV
III	Curative- Cisplatin on day 1+ plant extract (250mg/kg.,p.o)from day 6 to 15	5 th & 16 th	To assess curative effect of <i>Chrysanthemum indicum</i>
IV	Curative - Cisplatin on day 1+ plant extract (500mg/kg.,p.o)from day 6 to 15	5 th & 16 th	To assess curative effect of <i>Chrysanthemum indicum</i>
V	Prophylactic control - vehicle from day 1 to 10+ Cisplatin on day 11	15 th	To serve as prophylactic control for group VI
VI	Plant extract from day 1 to 10+ Cisplatin on day 11	15 th	To assess prophylactic effect of <i>Chrysanthemum indicum</i>
VII	Plant extract from day 1 to 10.	11 th	To observe effect of plant extract on kidney

On day 15, urine was collected with the help of metabolic cages and urine samples were subjected for estimation of urinary functional parameters. On day 16 animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture and were used for estimation of serum markers.

Assessment of renal function:

Nephroprotector activity was assessed by estimating Blood Urea Nitrogen (BUN) by DAM method⁹, Serum Creatinine (SC) by Jaffe.s Alkaline Picrate method,⁹ Urinary Total Proteins

(U_{TP}) by Turbidimetry method, Urinary Creatinine(Ucr) by Alkaline picrate Method⁹. Creatinine Clearance (Clcr) was calculated by using formula Creatinine clearance = Urinary creatinine x urinary volume/hr/ Serum creatinine. Lipid Peroxidation (LPO) in kidney tissue was estimated by following standard method¹⁰.

Histological studies:

Two animals from each group were sacrificed on day 15 or 16 and kidneys were isolated. The isolated kidneys were fixed in 10% neutral buffer formalin and processed to paraffin wax. Sections (5 microns) were stained with haematoxylin and eosin and were examined under light microscope.

Statistical analysis:

The statistical data was presented as mean \pm SEM. Parametric data which include all the biochemical parameters were analyzed using a paired 't' test for the paired data or one way analysis of variance (ANOVA) followed by a Dunnet multiple comparisons post test. A probability value of P<0.05 was considered as significant.

RESULTS AND DISCUSSION:

Preliminary phytochemical studies:

Phytochemical screening of the ethanol extract of flowers of *Chrysanthemum indicum* revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins and terpenoids. Column chromatography of the ethanol extract of flowers of *Chrysanthemum indicum*: Phytochemical investigation revealed the presence of Eicosan-1-ol (in 100% Petroleum ether eluent) and kikkanol-D-monoacetate (in 5:5 Petroleum ether and chloroform eluent). These compounds were characterized by comparing the spectral data (IR, ¹H NMR and Mass) with authentic samples. Earlier Kikkanol-D-monoacetate was reported from same species by gas chromatography. This is the first report mentioning the isolation of Kikkanol-D-monoacetate by column chromatography.

Pharmacological studies:

Animals which received plant extract alone (Group VII) for ten days, exhibited no change in serum markers level and urinary functional parameters. Hence, ethanol extract of flowers of *Chrysanthemum indicum* did not show any deteriorative effects on rat kidney.

Administration of Cisplatin 6mg/kg Bd.wt, i.p. caused elevation of Blood urea nitrogen (BUN), serum creatinine (SC), decreased creatinine clearance (Clcr), increased protein excretion in Urine, MDA levels.

In curative regimen, elevated levels of serum markers were significantly decreased with the administration of the ethanol extract of flowers of *Chrysanthemum indicum* in dose dependent manner.

In prophylactic regimen, animals treated with the ethanol extract of flowers of *Chrysanthemum indicum* at 500 mg/kg Bd wt, significantly decreased the levels of Blood urea nitrogen and serum creatinine compared to Group V (prophylactic control).

Group II animals showed high amount of protein excretion in Urine when compared with the normal control Group I animals. However administration of the ethanol extract of flowers of *Chrysanthemum indicum* reduced the urinary protein excretion caused by Cisplatin Animals which were pre-treated with extract for 10 days (Group VI) significantly reduced the Urinary proteins excretion to Group V animals.

Animals which received cisplatin alone exhibited decreased levels of Cl_{cr} when compared with normal animals. On oral administration of the ethanol extract of flowers of *Chrysanthemum indicum* showed significant increase in Cl_{cr} in dose related manner. In prophylactic regimen, animals which received extract exhibited significant increase in Cl_{cr} compared to Group V.

Animals treated with only Cisplatin showed increased levels of MDA compared to normal control animals and this effect was decreased significantly by the administration of the ethanol extract of flowers of *Chrysanthemum indicum*. In prophylactic regimen, animals belonging to Group VI exhibited significant decrease in MDA levels in comparison with prophylactic control (Group V).

Table-1: Effect of the ethanol extract of flowers of *Chrysanthemum indicum* on Cisplatin-induced Nephrotoxicity.

Group	Treatment	BUN(mg/dl)	SC(mg/dl)	LPO (nmol/mg tissue)
I	Normal(2% gum acacia)	29.75±0.21	0.92±0.005	11.09±0.06
II	Cisplatin (6mg/kg)(curative control)	60±0.32*	1.86±0.05*	15.57±0.27*
III	ethanol extract(250mg/kg)	44.75±0.20 ^a	1.42±0.05 ^a	13.5±2.4 ^a
IV	ethanol extract(500 mg/kg)	32.25±0.13 ^b	0.87±0.07 ^b	11.3±0.3 ^b
V	Cisplatin (6mg/kg)(prophylactic control)	61.0±0.10*	2.1±0.08*	14.77±0.26*
VI	ethanol extract(500 mg/kg)	27.75±0.83 ^c	1.0±0.07 ^c	11.0±0.9 ^c
VII	ethanol extract(500 mg/kg)	28.75±0.83 ^d	1.0±0.13 ^d	10.60±0.13 ^d

Each value represents the mean ± S.E.M from 6 animals in each group.

* p< 0.001 when compared with normal control group.

a: p< 0.001 when compared to control group.

b: p< 0.001 when compared to control group.

c:p<0.05 when compared with normal control animals.

d: p<0.001 when compared to normal group.

Table-2: Effect of the ethanol extract of flowers of *Chrysanthemum indicum* on Cisplatin-induced renal functional parameters

Group	Treatment	U _{TP} (mg/24hrs)	Cl _{cr} (ml/hr/100 gm bd wt)
I	Normal(2% gum acacia)	8.50±0.50	18.4±1.20
II	Cisplatin (6mg/kg)(curative control)	20.42±1.70 [*]	6.68±0.10 [*]
III	ethanol extract(250mg/kg)	12.06±1.23 ^a	13.9±1.4 ^a
IV	ethanol extract(500 mg/kg)	8.96±0.75 ^b	18.7±0.9 ^b
V	Cisplatin (6mg/kg)(prophylactic control)	18.52±1.70 [*]	7.05±0.12 [*]
VI	Ethanol extract (500 mg/kg)	9.50±0.45 ^c	18.3±0.59 ^c
VII	Ethanol extract (500 mg/kg)	8.15 ±0.48 ^d	18.3 ±0.59 ^d

Each value represents the mean ± S.E.M from 6 animals in each group.

* p< 0.001 when compared with normal control group.

a: p< 0.001 when compared to control group.

b: p< 0.001 when compared to control group.

c:p<0.05 when compared with normal control animals.

d: p<0.001 when compared to normal group.

Histological studies:

The sections of kidneys isolated from rats treated with cisplatin showed degenerative glomeruli, atropic glomeruli with hemorrhages, enlarged renal tubule with hemorrhages, enlarged tubules and vacuolization of renal tubule indicating renal toxicity.

The sections of kidneys isolated from rats treated with low dose of the ethanol extract of flowers of *Chrysanthemum indicum* (250mg/kg Bd wt) showed moderate degenerative change in glomeruli. The sections of kidneys isolated from rats treated with high dose of the ethanol extract of flowers of *Chrysanthemum indicum* (500mg/kg Bd wt) showed regenerative changes by reduction of picnotic nuclei and reduction in congestion of glomeruli. In prophylactic regimen (500mg/kg Bd wt) congestion of the glomeruli was reduced and regenerative changes in glomeruli indicating marked protection against Cisplatin-induced nephrotoxicity.

Previous reports on phytochemical studies of plants revealed the presence of terpenoids (indicumolide A, B, C), flavonoids (Luteolin 7-O-beta-glucouronide, Diosmetin7-O-beta-glucouronide). Above compounds have broad spectrum of biological activities including antioxidant activity²⁰. Hence, the possible mechanism by which ethanol extract had shown nephroprotector activity against Cisplatin-induced renal toxicity may be because of antioxidant principles like terpenoids and flavonoids.

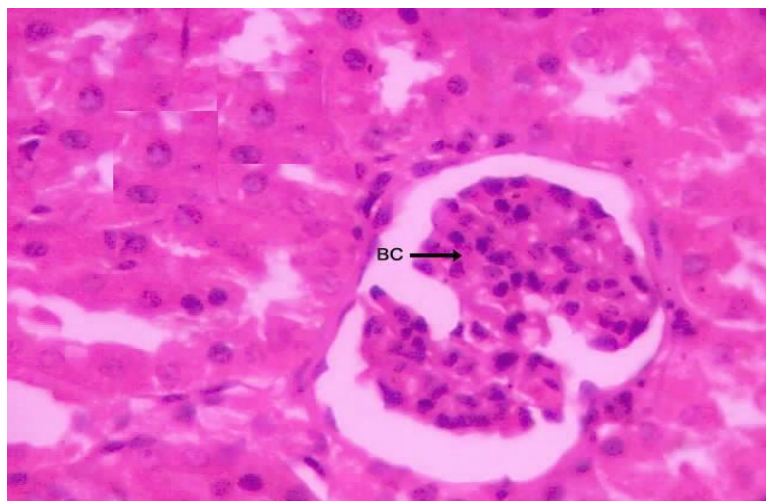


PLATE- 1: Section of rat kidney showing normal organization of Bowmans capsule.
BC-Bowmans Capsule.

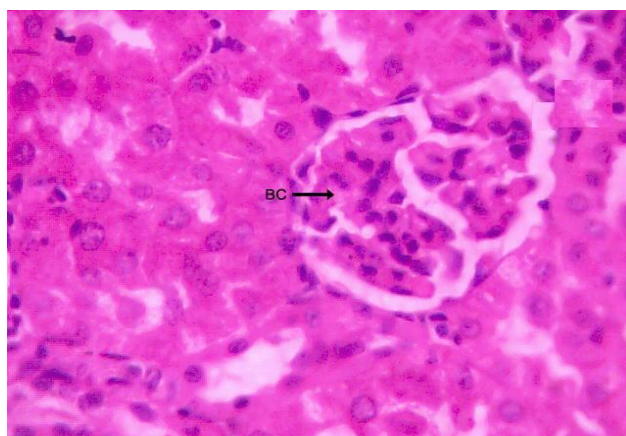


PLATE- 2: Section of rat kidney treated with ethanol extract of flowers of *Chrysanthemum indicum* (500mg/kg) BC- Bowmans Capsule

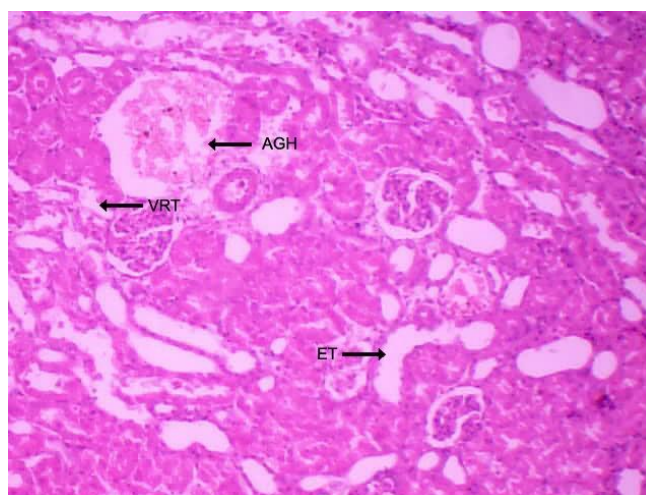


PLATE-3: Section of rat kidney treated with Cisplatin (6mg/kg, i.p.).AGH- Atrophic Glomeruli with Hemorrhages. ET- Enlarged Tubules. VRT- Vacuolization of Renal Tubule

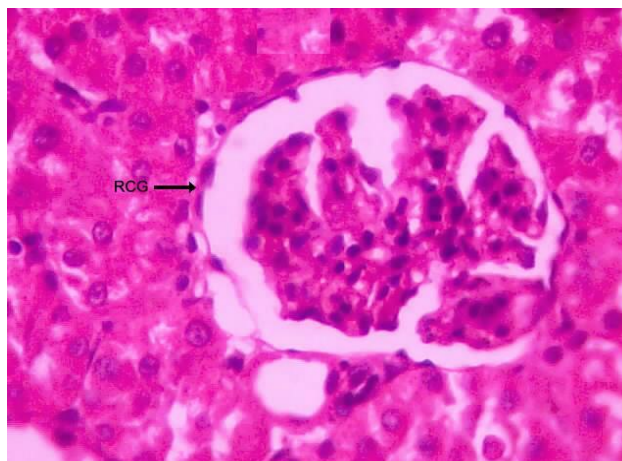


PLATE:4: Section of rat kidney treated with ethanol extract of flowers of *Chrysanthemum indicum* (250mg+Cisplatin) showing regenerative changes in glomeruli.

RCG – Regenerative changes in glomeruli

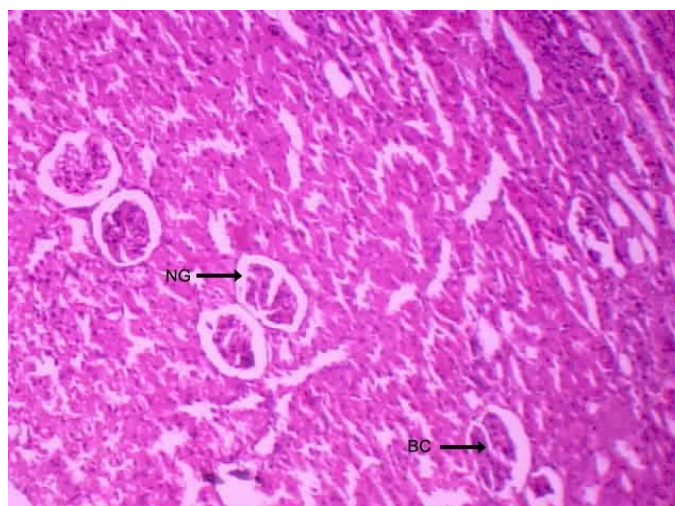


PLATE-5: Section of rat kidney treated with ethanol extract of flowers of *Chrysanthemum indicum* (500mg+Cisplatin) showing normal glomeruli.

NG- Normal Glomeruli,

BC- Bowmans capsule

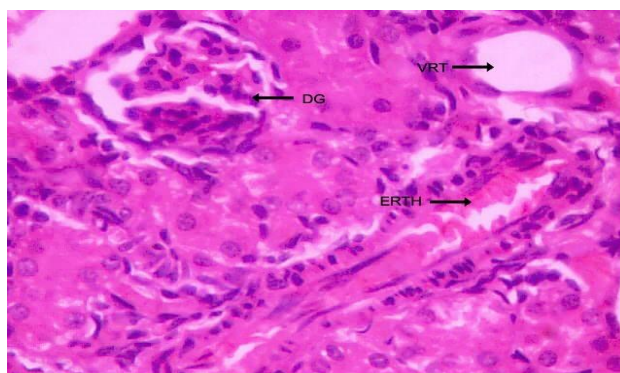


PLATE-6: Section of rat kidney treated with Cisplatin.ERTH- Enlarged Renal Tubule With Hemarages.

DG- Degenerative Glomeruli.

VRT- Vacuolization Of Renal Tubule

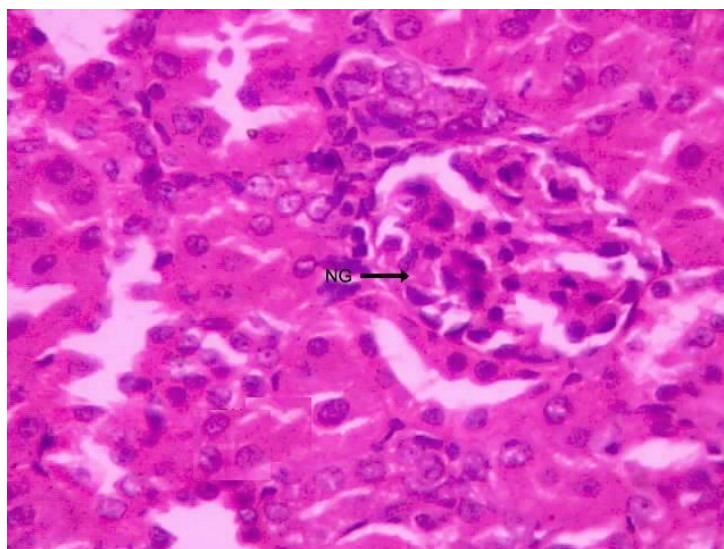


PLATE-7: Section of rat kidney treated with ethanol extract of flowers of *Chrysanthemum indicum* (500mg/kg) showing normal glomeruli.

NG- Nomal Glomeruli

CONCLUSION:

The results of this study demonstrated that the ethanol extract of *Chrysanthemum indicum* exhibited significant nephroprotector activity on Cisplatin-induced kidney damage in rats. However further investigation is needed to determine the exact phytoconstituents that are responsible for the nephroprotector activity of *Chrysanthemum indicum*.

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